Co-Regulation among genes and pathways that are responsive to low-dose ionizing radiation.

Matthew A. Coleman[1], Anya Krefft[1], Francesca Pearson[1], Leif E. Peterson[2], Jian Jian Li[3], Xiaowen Xin[1], Terrence Critchlow[1], Ilkay Altintas[4], Bertram Ludaescher[5] and Andrew J. Wyrobek[6].

[1]Biosciences, LLNL, Livermore, CA, 94550, [2]Departments of Molecular and Human Genetics and Medicine, Baylor College of Medicine, Houston, TX, 77030, [3]School of Health Sciences, Purdue University, West Lafayette, IN, 47907. [4]San Diego Supercomputer Center, University of California, San Diego La Jolla, CA 92093. [5]Department of Computer Science, University of California, Davis, CA 95616. [6]Life Science Division, Lawrence Berkeley National Laboratory, Berkeley, CA. 94706.

Contact information: coleman16@llnl.gov

Exposures of mammalian cells and tissues to ionizing radiation alters the transcriptional profile for hundreds of genes. Our approach to discovering the mechanisms of regulation of co-expressed genes is to link genomic sequence with bioinformatics databases to validate and characterize eukaryotic promoters responsive to varying levels of IR exposure. We developed a computational workflow that links microarray data with multiple bioinformatic resources to perform genome wide identification of basal promoter sequences. The computational workflow was applied to genome-scale expression microarray data to identify and validate gene regulatory elements that may control and differentiate aspects of cellular responses to ionizing radiation. Using expression data specific for low dose and adaptive response we found four sets of genes with the following IR response expression patterns: 1) Genes that are constantly upregulated at low dose, ~80 genes, 2) genes with increased expression with increasing dose, ~30 genes, 3) genes up or 4) down-regulated in cells that undergo the adaptive response (~150 genes). Cluster analysis found a pronounced pattern for transcript expression associated with increasing exposures of IR dose and contained 33 genes. A majority of these genes are known to be regulated by TP53. To better understand the IR responsive TP53 regulatory network we identified sequence level elements and transcription factor modules that were shared across the 33 genes. Three individual core modules (two or more transcription factor binding sites) were identified based on 5 of the 33 genes which were known to be regulated by TP53. All of these modules contained a TP53 binding site along with other known ionizing radiation responsive elements such as SP1 and CREB transcription factor binding sites. Additional shared modules were identified close to the start of transcription when selection was not biased by requiring the presence of TP53 binding sites. The most statistically significant modules of transcription binding elements were than used to predict novel IR responsive genes for which microarray data was not available. PCR was used to verify responses in two human lymphoblastoid cell lines for both low and high dose exposures. Chromatin immuno-precipitation experiments are being used to validate the presence or absence of TP53 in regulating the known and predicted IR responsive genes. These novel elements and modules define new IR responsive networks. Our data also suggest that proximal

promoter regulatory elements may act cooperatively with TRP53 to modulate the cells response at both high and low doses of IR. Our findings will help guide future experiments for understanding low dose IR regulatory mechanisms and provide the basis for identifying susceptibility regulatory factors involved in individual responses to low dose IR.

This work was performed under the auspices of the U.S. Department of Energy by the University of California, Lawrence Livermore National Laboratory under Contract No. W-7405-Eng-48 with funding from the Laboratory Directed Research and Development and DOE Low Dose Radiation Research programs.